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(54) STRESS DETERMINING METHOD AND DEVICE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method for determining stress on a subject by collecting saliva without exerting stress and simply measuring physiologically active substances in the saliva and a device used for the method.
SOLUTION: The activity of α-amylase in the saliva is taken as an index to determine stress load.

CLAIMS

[Claim(s)]
[Claim 1]A stress judging method of a test subject making into an index alpha-amylase activity which exists in a test subject's saliva.
[Claim 2]A stress judging method according to claim 1 which makes a sample saliva extracted without applying stress from a test subject.
[Claim 3]A stress judging method according to claim 1 or 2 of extracting said saliva from a test subject using capillarity.
[Claim 4]A stress judging method given in any 1 of claims 1-3 which make a reference value an alpha-amylase activity value in saliva of a test subject's resting period, and judge a grade of a size of stress according to a difference between a value at the time of measurement, and a reference value.
[Claim 5]A stress judging method of claim 4 which will judge a grade of a size of comfortable stress (eustress) if an alpha-amylase activity value in saliva at the time of measurement is larger than a reference value and it is smaller than a reference value in a size of unpleasant stress (distress).
[Claim 6]A stress judging method given in any 1 of claims 1-3 which judge a size of stress according to chronocline in aging of alpha-amylase activity in a test subject's saliva.
[Claim 7]A stress judging method according to claim 6 of judging a grade of a size of stress (eustress) comfortable from a size of negative chronocline for a grade of an unpleasant size of stress (distress) from a size of positive chronocline.
[Claim 8]In aging of alpha-amylase activity in a test subject's saliva, a value of the alpha-amylase activity in saliva of a resting period is made into a reference value, A stress judging method given in any 1 of claims 1-3 which judge a grade of a size of stress that subject reacts with the length of time until it returns to a reference value after adding mental or corporal stress.

[Claim 9]A stress determining device used for a stress judging method given in any 1 of claims 1-8 provided with a sensor part which makes it possible to measure alpha-amylase activity.

[Claim 10]The stress determining device according to claim 9 provided with a function in which saliva is extractable, according to capillarity.

[Claim 11]The stress determining device according to claim 10 which can insert a sensor part into the mouth and enables continuous measurement in extraction of saliva.

[Claim 12]At least two electrodes, a stress determining device given in any 1 of claims 9-11 provided with an electrochemical sensor containing a substrate film of alpha-amylase.

[Claim 13]The stress determining device according to claim 12 containing immobilized enzyme membrane which furthermore supports an imitation enzyme (conjugate enzyme).

[Claim 14]A stress determining device given in any 1 of claims 9-11 provided with a chemical sensor which recognizes coloring matter generated by the reaction of coloring matter generated by an alpha-amylase reaction at least or alpha-amylase, and an imitation enzyme (conjugate enzyme).

[Claim 15]The stress determining device according to claim 14 containing immobilized enzyme membrane which furthermore supports an imitation enzyme (conjugate enzyme).

[Claim 16]A stress determining device given in any 1 of claims 9-11 provided with a sensor using surface plasmon resonance which support a substrate or an antibody, a light emitting device, a photo detector, and a light reflection surface of alpha-amylase at least.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]This invention relates to the device used for the stress judging method which makes an index especially alpha-amylase activity in a test subject's saliva, and this method about the simple stress judging method.

[0002]

[Description of the Prior Art]various stress which people receive comes out, there is, and some are great as society becomes complicated in recent years. People who received various stress start various diseases, and the complicated therapy is demanded also of the ways of coping. In advance of the therapy of such stress, it is considered to occupy big importance in a medical field to judge the grade of stress.

[0003]either that the judgment method of old stress is mental to a living body, or a bodily pain -- although -- since not to be given at all was required, the available technique was limited considerably. Measuring a heart rate and blood pressure as a method generally used is mentioned. However, these methods were transient and were not able to be adopted as the judgment method of the stress which continues in the long run.

[0004]It is known well that neurotransmitter, such as adenocoriticotropic hormone, such as cortisol in blood, adrenaline, a noradrenalin, will serve as an index reflecting stress other than the physiological function which they originally have. However, in the ingredient in blood, since the concentration of hormone etc. is dramatically low, it needs a large-sized device for analysis, and there is not only a fatal fault that stress follows on extraction itself of blood, but measurement has taken the long time.

[0005]A chronic stress diagnosis which measures the cortisol concentration in saliva in the physiological active substance in saliva, for example (the patent No. 3108765 gazette), Stress assay which makes an index the adrenal sex steroid in saliva, and/or concentration of the metabolite (JP,11-38004,A), The method (JP,2000-131318,A) of analyzing ** and an unpleasant state of stress from the various index substances in the stress identifying method (JP,11-326318,A) which measures the dehydroepiandrosterone concentration in saliva and blood, urine, or saliva, etc. are proposed.

[0006]However, although an easy technique can be used for extracting the index in saliva and it is simple compared with the case where blood is extracted, Measurement of the physiological active substance which is an index has a complicated technique compared with the common reagent for diagnosis, using expensive drugs, and it is difficult to become common.

[0007]

[Problem(s) to be Solved by the Invention]There is a technical problem of this invention in providing the simple device which is in providing the stress judging method which can be judged that it is simple and easily, and is used for the judgment.

[0008]

[Means for Solving the Problem]In order for this invention persons to solve an aforementioned problem, when many things were examined wholeheartedly, paying attention to alpha-amylase in saliva, by a test subject who required stress, alpha-amylase activity found out a high thing and they reached this invention. That is, they are a stress judging method this invention's extracting saliva from a test subject, measuring alpha-amylase activity in this saliva, and judging a grade of a test subject's stress from this activity, and a stress determining device which has a sensor part in which alpha-amylase activity measurement is possible.

[0009]A stress judging method of a test subject, wherein this invention makes an index alpha-amylase activity which exists in 1. test subject's saliva.

2. Stress judging method given in the preceding clause 1 which makes sample saliva extracted without applying stress from test subject.

3. Stress judging method given in the preceding clause 1 or 2 which extracts said saliva from test subject using capillarity.

4. Stress judging method given in any 1 of the preceding clauses 1-3 which makes reference value alpha-amylase activity value in saliva of test subject's resting period, and judges grade of size of stress according to difference between value at time of measurement, and reference value.

5. Stress judging method of the preceding clause 4 of judging grade of size of comfortable stress (eustress) if alpha-amylase activity value in saliva at time of measurement is larger than reference value and it is smaller than reference value in grade of unpleasant size of stress (distress).

6. Stress judging method given in any 1 of the preceding clauses 1-3 which judges grade of size of stress according to chronocline in aging of alpha-amylase activity in test subject's saliva.

7. Stress judging method given in the preceding clause 6 which judges grade of size of stress (eustress) comfortable from size of negative chronocline for grade of unpleasant size of stress (distress) from size of positive chronocline.

8. In aging of alpha-amylase activity in a test subject's saliva, a value of the alpha-amylase activity in saliva of a resting period is made into a reference value, A stress judging method given in any 1 of the preceding clauses 1-3 which judges a grade of a size of stress that subject reacts with the length of time until it returns to a reference value after adding mental or corporal stress.

9. Stress determining device used for stress judging method given in any 1 of the preceding clauses 1-8 provided with sensor part which makes it possible to measure alpha-amylase activity.

10. A stress determining device given in the preceding clause 9 provided with a function in which saliva is extractable, according to capillarity.

11. A stress determining device given in the preceding clause 10 which can insert a sensor part into the mouth and enables continuous measurement in extraction of saliva.

12. At least two electrodes, a stress determining device given in any 1 of the preceding clauses 9-11 provided with an electrochemical sensor containing a substrate film of alpha-amylase.

13. Stress determining device given in the preceding clause 12 containing immobilized enzyme membrane which supports an imitation enzyme (conjugate enzyme) further.

14. A stress determining device given in any 1 of the preceding clauses 9-11 provided with a chemical sensor which recognizes coloring matter generated by the reaction of coloring matter generated by an alpha-amylase reaction at least or alpha-amylase, and an imitation enzyme (conjugate enzyme).

15. Stress determining device given in the preceding clause 14 containing immobilized

enzyme membrane which supports an imitation enzyme (conjugate enzyme) further.
16. A stress determining device given in any 1 of the preceding clauses 9-11 provided with a sensor using surface plasmon resonance which support a substrate or an antibody, a light emitting device, a photo detector, and a light reflection surface of alpha-amylase at least.
**, ** and others
[0010]
[Embodiment of the Invention](Extraction of saliva) It carries out, without extraction of saliva applying stress to a test subject's body or pneuma in this invention. As for a little especially output of saliva, what can be extracted in a short time without coming out, and giving a test subject stress since it is enough is desirable. To extraction of saliva, extracting using capillarity is preferred.
[0011]As an instrument which extracts saliva, the instrument (capillary tube) shown in drawing 1-A is illustrated. In order to enable suction of saliva by capillarity, three films of identical shape are used for the structure of this instrument, and it arranges the capillary tube formation board which formed the slot in the center, has the structure put by the superior lamella without a slot, and an inferior lamella from both sides, and constitutes a thin slot between an extraction mouth and the test paper. Since the plastic film has water repellence, it makes the included inside without the slot of two sheets have applied and dried the surface-active agent (for example, DK ester, cane-sugar fatty acid ester,: Dai-Ichi Kogyo Seiyaku Co., Ltd.) diluted with ethanol to 5%, for example generally.
[0012]Since it is necessary to consider it as a disposable type in order to insert an extraction instrument into the mouth, the material of construction may use only cheap polyester film. The thickness of the film per sheet shall be 30 micrometers, and joint formation is carried out by ultrasonic welding.
[0013]If the tip part 1 of the instrument shown in drawing 1-A is contacted to a test subject's tooth space, saliva will be extracted along the slot 2 from a tip part by capillarity. Saliva sinks into the test paper 3 and the saliva of about 1-10microl is extracted in several seconds.
[0014]Drawing 1 - To B, the mode which extracts saliva from a test subject is shown, saliva is extracted with the above-mentioned instrument, the above-mentioned instrument made to color or emit light with an alpha-amylase activity measurement reagent is inserted in an optical measurement meter, and alpha-amylase activity is measured. By inserting directly the sensor portion for alpha-amylase activity measurement into the mouth, it can measure continuously and it becomes possible to judge the stress received in the body in real time. It is preferred to have a doner site which can extract a little saliva in an instant using capillarity in the stress determining device by this invention.
[0015](Measurement of alpha-amylase activity) Although a publicly known measuring

method can already be used for enzyme activity measurement of the alpha-amylase used as the index of a stress judging, it is preferred to measure simple especially by the art of an enzyme sensor etc. for a short time. This enzymatic reaction product that made the alpha-amylase in saliva act on a substrate, and the imitation enzyme (conjugate enzyme) was made to specifically act as occasion demands, and was generated is detected, and alpha-amylase activity is measured. As said substrate, starch or oligosaccharide, these derivatives, etc. exist (622-626 pages of the clinical laboratory test manual amendment 31st edition versions).

[0016]The following reaction formula shows an example of the alpha-amylase activity measurement of this invention.

Alpha-amylase Glucosidase Starch -> malt sugar -> grape sugar ($C_6H_{12}O_6$)

glucose oxidase (GOD) -- $C_6H_{12}O_6+O_2$-> $C_6H_{10}O_6+H_2O_2H_2O_2$-> $2H^+ + 2e^- + O_2$ (current detection)

[0017]When said substrate is starch, make **-glucosidase act on the generated malt sugar, glucose OKIDAZE is made to act on the glucose generated further, and a sensor detects the generated electron or oxygen from hydrogen peroxide.

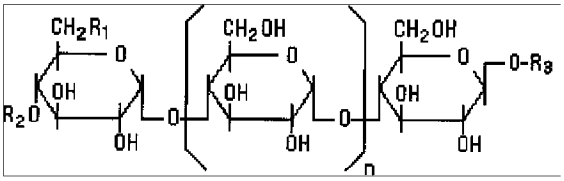
[0018]There is an electrode provided with the enzyme fixed film which has the structure shown in drawing 2 as a sensor used in this invention, for example. This sensor laminates a fixed glucose oxidase film and a fixed **-glucosidase film one by one on a hydrogen peroxide electrode. It acts on starch which is a substrate, and is decomposed into 2 - some maltooligosaccharides, and glucose generates the alpha-amylase in saliva by operation of **-glucosidase further. Glucose generates hydrogen peroxide by operation of glucose oxidase, and measures this hydrogen peroxide in an electrode. Each substance was specified with each sign under drawing 2.

[0019]As an electrode, the enzyme FET sensor, an enzyme photo-diode, an OPURODO type sensor, or a surface plasmon type sensor other than an oxygen electrode, etc. can be used. It can miniaturize and these sensors can measure alpha-amylase activity by choosing portable size at the spot which extracted saliva in a short time.

[0020]As a substrate of alpha-amylase, it is a maltooligosaccharide which comprises a glucose unit other than starch, And the nonreducible end glucose is embellished with substituents other than glucose, the maltooligosaccharide etc. which have combined detectable coloring matter etc. in the position of the 1st place of/or reducing end glucose may be used. Specifically, the ornamentation maltooligosaccharide which consists of the following general formula can serve as a substrate of alpha-amylase.

[0021]

[Formula 1]



[0022]R_1 in a formula and R_2 may be embellished by the hydrogen atom or the protective group, respectively. Although a protective group is not limited exceptionally, for example The low-grade alkyl group of unsubstituted or substitution, A low-grade alkoxyl group or a phenyl group, an azido group, a halogen atom, an N-monoalkyl carbamoyloxy group, It is alkyl, an arylsulfonyloxy group or an alkyloxy group, alpha-glucosyl group, an alpha-malto sill group, and beta-galactosyl group, R_1 and R_2 may be constructing the bridge mutually, and it may have a substituent further in this crosslinking group. R_3 is a hydrogen atom or a signal generating group (suitably color-enhancing aromatic group), for example, the basis which can detect a signal optically, and n is 0-5. At the above-mentioned formula, $-OR_3$ is beta to the 1st place of reducing end glucose. - It is alpha although it joins together. - It may join together.

[0023]More specifically as an example of such a maltooligosaccharide, It is usable in p-nitrophenyl maltopentaose, 2-chloro-4-nitrophenyl maltopentaose, 2-chloro-p-nitrophenyl maltotetraose, and 4-nitrophenyl benzyl-alpha-malto penta OSHIDO etc.

[0024]It is also possible to make **-glucosidase act on a maltooligosaccharide generated by alpha-amylase from substrates, such as starch, to make glucose dehydrogenase act on generated glucose under existence of NAD, and to measure NADH in measurement of alpha-amylase activity.

[0025](Reaction condition) Saliva concentration by which measurement of the alpha-amylase activity of this invention was extracted is given to measurement as it is, without thinning. substrate concentration -- the very thing -- the publicly known optimum amount is used. Usually, it is prepared so that it may become about [0.05mM-1M] concentration. Although reaction temperature in particular is not limited, it is about 25-40 ** preferably. Although reaction time is enough in 1 to about ten minutes, it depends for it on a kind of a substrate and conjugate enzyme. Although reaction optimal pH in particular is not limited, it may be adjusted to pH 6-8 with suitable buffer solution by request. An activator of alpha-amylase publicly known for promotion of a reaction may be used by request.

[0026](Stress determining device) A stress determining device of this invention has a sensor part in which alpha-amylase enzyme activity measurement is possible. With a sensor which can measure alpha-amylase activity in saliva used in this invention simple. (1) a substrate of at least two electrodes and alpha-amylases, and necessity -- an imitation enzyme (conjugate enzyme) (**-glucosidase.) An electrochemical sensor containing **-glucosidase, glucose oxidase, etc., (2) Coloring matter generated by an alpha-amylase reaction from an alpha-amylase substrate (for example, p-nitrophenyl malto TORIOSHIDO) at least (for example, p-nitrophenol), From an alpha-amylase substrate (for example, starch) to or alpha-amylase. To hydrogen peroxide generated by the reaction of **-glucosidase and glucose oxidase. a chemical sensor which recognizes

coloring matter which peroxidase, 4-aminoantipyrine and aniline, or phenols are made to react, and is generated, and (3) -- a sensor using surface plasmon resonance etc. which support an alpha-amylase substrate, a light emitting device, a photo detector, and a light reflection surface at least are contained. As for an imitation enzyme (conjugate enzyme), being fixed is more preferred.

[0027]Structure of electrochemical sensors is shown in drawing 12. The electrode 1 and the electrode 2 are formed in a base surface formed with material which has insulation. A resin film or carbon can be used as construction material of a base. Even if it uses which method for formation of these electrodes at etching, screen-stencil, or adhesion fixing of an electrode sheet, it is uninfluential in an effect of this invention.

[0028]A substrate film which fully contained a substrate to amylase is formed in a tip upper surface of these electrodes 1 and 2. High molecular compounds, such as protein harmless to carboxymethyl cellulose (CMC), cellulose, and other human bodies, are used for a substrate of this substrate film. And in order that a portion which is not covered by a substrate film may prevent a short circuit by ******, covering for protection is provided.

[0029]If this electrochemical sensor is inserted into the mouth, as indicated to measurement of alpha-amylase activity, alpha-amylase contained in saliva will promote disassembly of a substrate of a substrate film, a predetermined chemical reaction will progress, and current according to alpha-amylase concentration will be measured as the result. That is, if this electrochemical sensor is used, it will become possible to measure alpha-amylase activity in saliva continuously. Here, a member which forms it in such electrochemical sensors can be constituted only from that by which living body safety was confirmed, and its safe top is also satisfactory at all.

[0030]Next, structure of a sensor of using surface plasmon resonance is shown in drawing 13. This sensor comprises a glass substrate in which a metal thin film was formed in surface one side, light sources, such as LED and laser, a photo detector and prism formed in a field in which a metal thin film of a glass substrate is not formed, and ligand fixed on a metal thin film. In measuring alpha-amylase activity, it fixes a substrate or an antibody as this ligand.

[0031]If an extracted saliva sample reaches a sensor surface via a channel, a position of light which a rate of optical refraction changes according to this alpha-amylase activity, and is received with a photo detector as a result will change. Alpha-amylase activity in saliva can be measured from this difference. Since only a chemical which exists from the first in the living body can be used for ligand fixed on a metal thin film by this sensor, the channel can also insert not indispensable requirements but this sensor into the mouth directly.

[0032](A judgment method of stress) Both corporal stress and psychological stress are included with stress in this invention. A stress diagnosis of this invention is judged by the amount of alpha-amylases in saliva, especially enzyme activity.

[0033]Alpha-amylase activity in saliva extracted to a test subject's resting period is specifically measured, the activity value is recorded and memorized, and it is considered as a reference value. Alpha-amylase activity in a test subject's arbitrary conditions is measured to the appropriate back, and it compares with a reference value recorded and memorized to a resting period. It judges with having received unpleasant stress (distress), if enzyme activity is larger than a reference value, and if small, it can judge with having received comfortable stress (eustress). Received stress is also large and a grade of stress received in the body or pneuma can also be judged, so that a difference with a reference value is large.

[0034]Change of temporal stress can be caught by measuring alpha-amylase activity continuously. If unpleasant stress is received, alpha-amylase activity in saliva will go up. A grade of a size of stress can be judged with a size of positive chronocline in this case. Conversely, since the enzyme activity of alpha-amylase falls when having received comfortable stress, it appears as negative chronocline and a grade of the size can be judged similarly.

[0035]Alpha-amylase activity can be measured temporally, enzyme activity change by arbitrary stress added in measuring time can be caught, and a grade of a size of stress can be judged from a size of time and change until it returns to a value (reference value) in front of a stress burden.

[0036]

[Example]Hereafter, this invention is explained in detail using an example.

[0037]

[Example 1] Using four test subjects who elected arbitrarily, the cold pressor test was carried out and the judgment of stress was tried. With a cold pressor test, one hand is dipped in the container into which ice water went till the place of a wrist for 30 seconds, a hand is taken out from a container after that, and it rests for 30 seconds. It is a test to which stress is applied by repeating this 7 times.

[0038]A result is shown in drawing 3 - 6. The horizontal axis shows the lapsed time after starting a cold pressor test, and extracted saliva several times during operation of a cold pressure, and after operation. The vertical axis expresses the alpha-amylase activity value in the extracted saliva. The enzyme activity of alpha-amylase goes up by a cold pressure, i.e., stress, and it turns out after the end of a cold pressure that it is falling.

[0039]Although the activity value of alpha-amylase and the method of change change with test subjects, the grades which this senses as stress by a test subject differed, that is, the difference in sensibility has appeared. Compared with the test subject A, it turns out by the test subject B that the grade of the stress received from a cold pressor test is small. It thinks, also when a pleasant sensation (joy) is sensed and alpha-amylase activity falls rapidly after the end of a cold pressure, since [to say] a pressure can be stopped. In the test subject C, unlike other test subjects, the activity value of alpha-amylase has returned even to the value at the time of a test start immediately after

the end of a cold pressure in this example. It is shown that a pleasant sensation by having been wide opened from stress is acting.

[0040]

[Example 2] Using the test subject of a binary name who elected arbitrarily, the KUREPE phosphorus test was carried out and the judgment of stress was tried. A KUREPE phosphorus test is the written examination currently devised as a way method of psychological tests, and is a stress burden examination to which follow a test subject and addition is made to carry out to him using the paper with which the numerical value of a single figure is located in a line and which gives mental anguish.

[0041]A result is shown in drawing 7 - 8. It is an activity value of alpha-amylase on the lapsed time from a KUREPE phosphorus test start, and a vertical axis like drawing 3 - 6 at a horizontal axis. Here, the activity value of alpha-amylase rose by a KUREPE phosphorus test, i.e., stress, like Example 1, and after the KUREPE phosphorus test was completed, the activity value of alpha-amylase fell.

[0042]

[Example 3] Using one test subject who elected arbitrarily, the mirror drawing test was carried out and the judgment of stress was tried. A mirror drawing test is a stress burden examination on which a predetermined course is made to draw with a pen, looking at the image which stood the partition so that its hand could not be seen directly, and was reflected in the mirror. It was made to draw with a pen in an exam, using the paper with which the line of the double star shape was printed, so that between lines may not be protruded. When overflowing, the penalty of redoing was given and it was made to continue for 5 minutes from a start spot once again.

[0043]A result is shown in drawing 9. It is an activity value of alpha-amylase on the lapsed time from a mirror drawing test start, and a vertical axis at a horizontal axis. The activity value of alpha-amylase rose by a mirror drawing test, i.e., stress, like Examples 1 and 2, and after the mirror drawing test was completed, the activity value of alpha-amylase fell.

[0044]

[Example 4] Using the test subject of a binary name who elected arbitrarily, the puncture test was carried out and the judgment of stress was tried. This is a test which gives stress by carrying out the puncture of the finger tip oneself using the needle for blood sugar determination.

[0045]A result is shown in drawing 10 - 11. In drawing 10, the after-operation alpha-amylase activity value rose the puncture. In drawing 11, the alpha-amylase activity value rose with directions of the puncture, and the alpha-amylase activity value had already risen at the time of operation of a puncture.

[0046]

[Example 5] The judgment of the relaxation effect after KUREPE phosphorus test implementation was tried using one test subject who elected arbitrarily. This is finding a

relation with the relaxation effect by having a posture of a seating position or the supine position taken, or having a massage received after carrying out the KUREPE phosphorus test which is psychological stress load, and judges the size of comfortable stress (eustress).

[0047]A result is shown in drawing 14 -16 and Table 1. In drawing 14, when I had a seating position taken after KUREPE phosphorus test implementation, the alpha-amylase activity value descended slowly. In drawing 15, when I had the supine position taken after KUREPE phosphorus test implementation, the alpha-amylase activity value descended early compared with the seating position. In drawing 16, when I had a massage received after KUREPE phosphorus test implementation, the alpha-amylase activity value descended promptly. In order to see the rise of an alpha-amylase activity value, and aging of descent, the chronocline of the alpha-amylase activity value was searched for in drawing 14, and 15 and 16, but the KUREPE phosphorus test showed negative chronocline for positive chronocline by relaxation. Although the above result was summarized in Table 1, in Table 1, time until an alpha-amylase activity value returns to the value of a resting period was shortened by comfortable stress (eustress), and the chronocline in aging of an alpha-amylase activity value presented rapid negative inclination.

[0048]

[Table 1]

	アミラーゼ濃度 (U/l)		時間 (min.)		勾配 (U/(l·min.))		
	安静時	最大値	最大値まで	復帰まで	最大値	最小値	ピーク to ピーク
座位	250.56	649.27	8	29			
仰臥位	334.08	576.59	12	24	50.02	−33.19	83.21
仰臥位＋マッサージ	292.32	693.24	13	20	49.24	−95.07	144.31

表1　ストレスおよびリラクゼーションによるα－アミラーゼ活性値の変動

[0049]

[Effect of the Invention]As described above, according to this invention, the art of judging stress on that spot for a short time was able to be established by extracting saliva, without applying stress and measuring the alpha-amylase activity in saliva.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1]A is an exploded view showing the chemical sensor (instrument) which has a saliva doner site and a chromogen, and B is a figure showing the mode which extracts saliva from a test subject and inserts this instrument in a measurement meter. ** in a figure -- a tip part and ** -- a slot and ** -- the test paper and 1, as for a measurement hole (1.6 mm in diameter), and 2, a superior lamella (polyester) and 3 mean a capillary tube formation board (polyester), 4 means an inferior lamella (polyester), and 5 means a surface-active agent.

[Drawing 2]It is a figure showing the structure of an enzyme sensor of having the immobilized enzyme membrane used for alpha-amylase activity measurement. The meaning of each sign was shown in the figure.

[Drawing 3]

[Drawing 4]

[Drawing 5]

[Drawing 6]It is a table showing change of the alpha-amylase activity value by a cold pressor test.

[Drawing 7]

[Drawing 8]It is a table showing change of the alpha-amylase activity value by a KUREPE phosphorus test.

[Drawing 9]It is a table showing change of the alpha-amylase activity value by a mirror drawing test.

[Drawing 10]

[Drawing 11]It is a table showing change of the alpha-amylase activity value by a puncture test.

[Drawing 12]It is a figure showing the structure of an electrochemical sensor.

[Drawing 13]It is a figure showing the structure of a sensor of using surface plasmon resonance. The ligand and the white round head by which reverse Y character white was fixed mean a substance under test among a figure.

[Drawing 14]

[Drawing 15]

[Drawing 16]It is a table showing change of the relaxation effect after KUREPE phosphorus test implementation.